STS-85 Continues PCAM Experiments

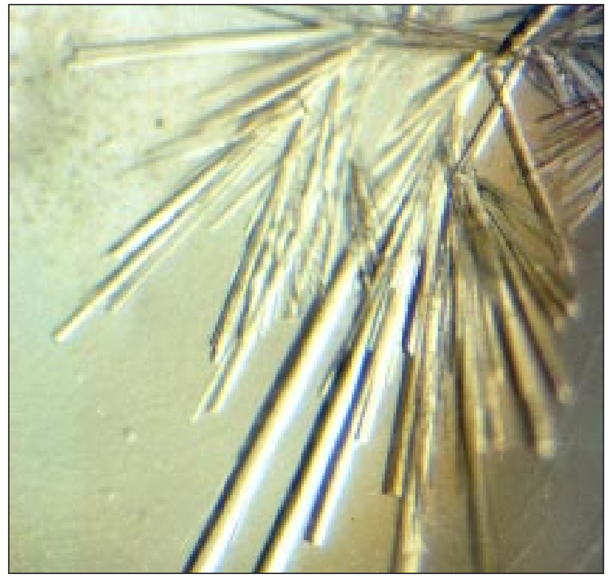
Apparatus to make 5th flight in space

Protein Crystal Growth Program

Microgravity Research Program Office

The STS-85 Space Shuttle mission, set for launch in August 1997, will carry several Protein Crystallization Apparatus for Microgravity (PCAM), sponsored by Marshall Space Flight Center (MSFC), to expand research into the structure and nature of protein molecules and other macromolecules. The PCAM has flown on previous Shuttle missions. In each case, they performed as planned and yielded intriguing results.

Proteins are important, complex biological chemicals which serve a variety of functions in all living organisms. Determining their molecular structures will lead to greater understanding of their functions in living organisms. Many proteins can be crystallized and their molecular structures determined through analysis of those crystals by X-ray crystallography. Crystals grown in the 1-g environment of Earth often have internal defects that make such analysis difficult or impossible. As demonstrated on Space Shuttle missions since 1985, some protein crystals grown in space are larger, and have fewer defects, than their Earth-grown counterparts.



Neeedlelike crystals of pike fish parvalbumin grown in one of the PCAM trays during MSL-1 (STS-94, July 1997) mission produced the first ultra-high resolution PCG structures from microgravity. Pike parvalbumin is used in fundamental biochemistry studies.

These experiments are managed by the MSFC Microgravity Research Program Office, Biotechnology Project Office. The program manager is Ron Porter. Key personnel are listed on the back page.

Protein crystallization methods

Hardware

PCAM (Protein Crystallization Apparatus for Microgravity)
STES (Single-locker Thermal Enclosure System)

PCG apparatus aboard STS-85 will grow crystals by vapor diffusion in the Protein Crystallization Apparatus for Microgravity (PCAM). Vapor diffusion is based on the drop method used extensively on Earth and in the NASA PCG program. In vapor diffusion, almost half the water from a protein droplet moves from the droplet to a reservoir solution. As the droplet becomes more concentrated, crystallization starts. Using lessons from earlier missions, PCAM will provide a means for growing larger quantities of crystals with less crew monitoring.

▶ Protein Crystallization Apparatus for Microgravity (PCAM)

The PCAM array on STS-85 comprises 10 cylinders containing 630 samples. Six PCAM cylinders will be contained inside a STES unit which will maintain temperatures at 22 ˚C; another four cylinders will be stowed in a locker at ambient temperature.

The basic PCAM design places a protein sample drop in a small well surrounded by an absorbent reservoir. A synthetic rubber (elastomer) seal isolates the drop from the reservoir except during crystal growth while in space.

A single, full-length PCAM cylinder is 81 mm (3.2 in) in diameter and 381 mm (15 in) long. Each cylinder contains nine trays held in position by guide rods and separated from each other by an actuator plate pushed by springs. The trays are sealed by an elastomer which uses adhesive to bind it to the upper face of the tray around the wells. A

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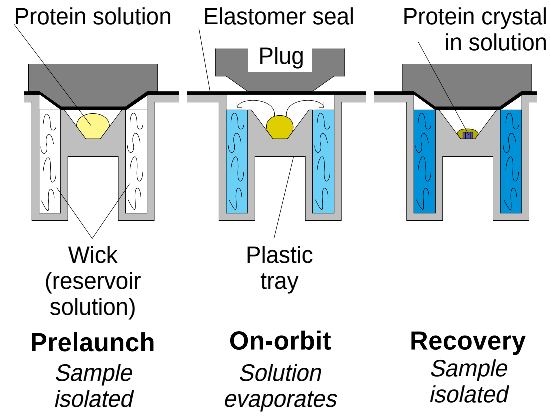
Solution droplets are placed in wells at the center of each of the seven tiny cups in a PCAM tray. The “moats” around the wells are filled with absorbent material, and an elastomer layer seals the top of the tray.

tray, in turn, holds seven sample wells, each surrounded by a donut-shaped reservoir which absorbs the precipitant solution.

From laboratory preparation until the start of the experiment in space, the elastomer seal is pressed by the actuator plate plate so it isolates each samples from rest of its chamber. To start the experiment, an astronaut opens the front of the STES and, using socket wrench, rotates a shaft on the end of the cylinder clockwise. This lets the seal retract and allow vapor diffusion to start. When all six cylinders are activated, the astronaut closes the STES. Before the end of the mission, the astronaut rotates the shafts counterclockwise to reseal the samples before return to Earth. The samples remain sealed until they are returned to the laboratory for analysis.

PCAM trays have been flown on several missions to date and have demonstrated that this is an effective means for screening large quantities of proteins for detailed study later. PCAM was developed at the MSFC Laboratory for Structural Biology.

Schematic of PCAM operation



Note: Actual size. Crystal drawing and colors are representative, not definitive.

Single-locker Thermal Enclosure System (STES)

Growing protein crystals requires a well-controlled thermal environment. The PCAM units will be housed in Single-locker Thermal Enclosure Systems (STES). Each STES takes the volume of a single middeck locker—51.6 x 46 x 27.2 cm (20.3 x 18.1 x 10.7 in)—and supports approximately 12.7 kg (28 lbs) of experiment apparatus.

STES has a 4-button keypad with a small data display and a 30-day, 272-kilobyte data logger. Temperature is controlled by forced-air cooling and by thermal-electric units which use the Peltier effect to move heat. STES temperatures will be maintained at 22 ˚C during the mission. (The Peltier effect produces or removes heat by an electric current flowing through the junction of two disimilar metals, depending on the direction of the current.)

On STS-85, one STES will contain a total of 6 PCAM cylinders (378 samples).

Candidate samples

Protein samples for crystallization in space are selected by a committee chaired by the PCG project scientist, Space Science Laboratory, MSFC. Samples are then evaluated and approved by NASA toxicology and safety offices.

As a point of comparison, the molecular masses of proteins range from about 890 to 2,200 times that of ordinary sugar, a relatively simple organic compound which is easily crystallized.

Candidate proteins for PCAM include respiratory syncytial antibody (a key factor in a severe lung disease of children), augmentor of liver regeneration (involved in the regrowth of damaged livers), human serum albumin (a key protein which carries chemicals through the bloodstream), human cytomegalovirus assemblin (a factor in CMV replication), human antithrombin III (a blood clotting factor), and neuorphysin vasopressin complex (involved in the control of blood pressure).

Key personnel, STS-85 PCAM experiments

	Principal Investigator Guest Investigators	Project Manager	Project Scientist
PCAM	Dr. Daniel Carter New Century Pharmaceuticals	Keith Higginbotham MSFC Biotechnology Project Office	Dr. Marcus Vlasse MSFC Space Sciences Laboratory
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